SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL EVALUATION OF SOME NEWER SUBSTITUTED BENZIMIDAZOLE DERIVATIVES

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ABSTRACT

α-phenylenediamine dihydrochloride (1) when reacted with different carboxylic acid derivatives yielded 2-substituted benzimidazole derivatives (2 a-i) which on methylation afforded 1-Methyl-2-substituted benzimidazole derivatives (3 a-i), and on acetylation yielded 1-Acetyl-2-substituted benzimidazole derivatives (3 j-o). The synthesized compounds were elucidated by physical and spectral data and screened for in vitro antibacterial activity against two gram positive (Staphylococcus aureus, Baccilus subtilis) and two gram negative bacterial strain (Pseudomonas aeruginosa, Escherichia coli) and invitro antifungal activity against Candida albicans, Aspergillus niger.

KEY WORDS: Benzimidazole, antibacterial and antifungal activity.

1. INTRODUCTION

Benzimidazoles are an important group of heterocyclic compounds which are biologically active. The benzimidazole nucleus is of significant importance in medicinal chemistry. In light of the affinity they display towards various enzymes and protein receptors, medicinal chemists would certainly classify them as privileged ‘sub-structures’ for drug dosing. The incorporation of the nucleus is an important synthetic strategy in studies of antimicrobial drug discovery.

Benzimidazoles derivatives have a broad antifungal spectrum (Bhaskar, 2007) and display their antifungal activities by blocking the polymerization of α and β-tubulin subunits. Antitubulin agents, especially benzimidazoles, disrupt microtubule function in eucaryotic organism such as fungi, protozoa and helminths. The benzimidazole still remains as one of the most versatile class of compounds against microbes and, therefore, is useful substrates for further molecular exploration.

Literature survey revealed that among the benzimidazole derivatives, 2-substituted ones are found to be pharmacologically more potent and hence the synthesis of 2-substituted benzimidazoles are the potential area of research. Benzimidazole show different pharmacological activities like antibacterial (Agh-atabay, 2003; Ansari and Ial, 2009), antifungal (Bhaskar, 2007; Goudgaon, 2004), anthelmintic (Dahiya and pathak, 2007; Mavrova, 2006; Luis, 2009), anti-inflammatory and analgesic (Dubey, 2007), antiulcer (Patil, 2010).

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2. MATERIALS AND METHODS

The melting points were determined in open capillaries and are uncorrected. The temperatures were expressed in °C and are uncorrected. The IR spectra of compounds were recorded on Perkin-Elmer infrared-283 FTIR spectrometer by KBr pellet technique and are expressed in cm⁻¹. 1H-NMR spectra were recorded on Bruker DRX-300 (300 MHZ, FT NMR) spectrophotometer using TMS as an internal standard, DMSO-d₆ as solvents. Mass spectrum was obtained using MS (Micromass quattroII) under Electron Spray Ionization (ESI) technique and elemental analysis was performed using Elemental Vario EL III, Carlo- Erba 1108. TLC was performed to monitor the reactions and to determine the purity of the products on a precoated aluminum plates using 30% methanol in chloroform as a mobile phase.

General methods for the synthesis

Synthesis of 2-substituted benzimidazole derivatives (2a-i)

Equimolar amounts (0.01 M) of α-phenylenediamine dihydrochloride, carboxylic acid derivative and water (4-5 ml) were added to the round bottom flask and the reaction mixture was refluxed for 3-4 h. The flask was then removed, cooled at room temperature and concentrated ammonia solution was added to the reaction mixture slowly with constant stirring until the mixture was just alkaline to litmus. Then the crude benzimidazole derivatives were filtered off, dried and recrystallised by q. ethanol.
Procedure for methylation of 2-substituted benzimidazole derivatives (3a-i)

Equimolar amounts (0.01 M) of 2-substituted benzimidazole derivatives, methyl iodide and potassium carbonate were transferred to a 100 ml beaker. 5 ml DMF (dimethyl formamide) was added. The reaction mixture was thoroughly mixed and kept in microwave oven. Microwave irradiation was done at 350 Watt for 3-4 minutes with intermittent cooling and mixing after every 1 minute. The completion of the reaction was monitored through TLC using 30% methanol in chloroform. The reaction mixture was cooled at room temperature. The ice cold water was added to the reaction mixture, a precipitate was immediately formed which was then filtered, washed with water, dried and recrystallised with aq. ethanol.

Procedure for acetylation of 2-substituted benzimidazole derivatives (3j-o)

Equimolar amounts (0.01 M) of 2-substituted benzimidazole derivatives, acetyl chloride and pyridine (0.9 ml) were transferred to a 250 ml round bottom flask. 10-15 ml of DMF was used as a solvent. The reaction mixture was refluxed on water bath for 4-5 h. the completion of reaction was monitored through TLC using 30% methanol in chloroform. The reaction mixture was cooled at room temperature and ice cold water was added, a precipitate was immediately formed which was then filtered, washed with water, dried and recrystallised with aq. ethanol.

Antibacterial activity

The antibacterial activity of newly synthesized compounds were tested by paper disc diffusion method using nutrient agar medium against following microorganism: S. aureus, B. subtilis, (Gram positive) and E. coli, P. aeruginosa, (Gram negative).

In the paper disc-diffusion method, paper disc impregnated with compounds dissolved in DMF at concentration 50 μg ml⁻¹ were used. Disc impregnated with DMF were used as solvent control for antibacterial activity because of free solubility of test compounds. The microorganism culture was spread over nutrient agar media in petri dishes, and then the disc impregnated with the solution was placed on the surface of the media inoculated with the bacterial strain. The plates were incubated at 35°C for 24 hrs for bacterial cultures. After incubation, the zones of inhibition around the disc were observed. The zones of inhibition indicate that the compounds inhibit growth of microorganism. Each testing is done in triplicate. Ciprofloxacin at conc. 50μg ml⁻¹ were used as standard drug for antibacterial activity.

Results were interpreted in terms of diameter (mm) of zone of inhibition. The results of antibacterial studies are presented in table 2.

Antifungal activity

Antifungal activity of newly synthesized compounds was tested on following fungal strains: A. niger and C. albicans. Disc diffusion method was performed using nutrient agar media.

In the disc-diffusion method, disc impregnated with compounds dissolved in DMF at concentration 50 μg ml⁻¹ were used. Disc impregnated with DMF were used as solvent control for antifungal activity because of free solubility of test compounds. The microorganism culture was spread over nutrient agar media in petri dishes, and then the disc impregnated with the solution was placed on the surface of the media inoculated with the fungal strain. The plates were incubated at 25°C for 48 hrs for fungal strains. After incubation, the growth inhibiting zones around the disc were observed. Growth inhibiting zone indicate that the compounds inhibit growth of microorganism. Each experiment is done in triplicate. Griseofulvin at concentration 50 μgml⁻¹ was used as standard drug for antifungal activity. Results were interpreted in terms of diameter (mm) of zone of inhibition. The results of antifungal studies are presented in table 2.

3. RESULTS AND DISCUSSION

A total of 15 compounds belonging to 2-substituted benzimidazoles were synthesized and showed remarkable activity in comparison to standard drug (Ciprofloxacin and Griseofulvin). TLC confirmed the purity of the synthesized compounds. The structure elugdication was done by interpreting FTIR, ¹HNMR, MASS spectra and Elemental analysis. It is deduced from the data that the compounds having electron withdrawing substitutions i.e. NO₂, Cl tends to enhance the antimicrobial activity.

The newly synthesized compounds (3a-o) were screened for antibacterial and antifungal activities respectively. Antibacterial activity was screened against Gram positive and Gram negative bacteria and antifungal activity by paper disc diffusion method, using nutrient agar medium. The results revealed that newly synthesized compounds 3f, 3g, 3l, 3m and 3n were found to be potent against E. coli; 3f, 3g, 3h, 3i, 3k and 3m against B. subtilis; 3g, 3j, 3l and 3o against P. aeruginosa, 3d, 3h, 3k, 3l and 3m against S. aureus and in antifungal activity 3f, 3g, 3i, 3l and 3o were found to be potent against C. albicans while 3f, 3i, 3l, 3m and 3o were found to be potent against A. niger.
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